CONTROL OF FLASHING IN FIREFLIES. II. ROLE OF CENTRAL NERVOUS SYSTEM

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In our preceding paper (Buck and Case, 1961, hereinafter referred to as "FF-I") we showed that many responses of the firefly lantern to electrical stimulation are analogous to those of conventional neuro-effectors such as striated muscle. In that paper we did not attempt to distinguish between central and peripheral nervous mediation in the excitation process. In the present investigation we have explored the roles of brain and cord more specifically. Some of the data have been summarized in abstracts (Case and Buck, 1958 and others cited in FF-I). We acknowledge with pleasure the assistance of Mr. Frank Hanson in the experimental work, and of Dr. Seymour Geisser in the statistical analysis in Section 4C.

Materials and Methods

We studied adults of the lampyrid fireflies *Photinus pyralis* from Maryland and Iowa, *Photinus marginellus* and *Photinus consanguineus* from Woods Hole, *Photinus punctulatus* from Iowa, and the common photurid (*Photuris versicolor*?) from all three localities. Males were used exclusively except for *Photuris*.

The lantern consists of two thin photogenic organs, one in abdominal segment 6 and one in 7. Each organ occupies most of the ventral surface of its segment, just inside the transparent cuticle.

The central nervous system comprises brain and suboesophageal ganglion in the head, and a ventral cord consisting of three ganglia in the thorax and seven in the abdomen. The gross innervation of the lantern is derived from abdominal ganglia 4–6, each segmental photogenic organ receiving nerves from at least two ganglia (Hanson, 1962).

Stimuli were delivered by Grass S-4 stimulators via r-f isolation units and electrode pairs of 0.005-inch bare silver wire. The wires were placed 1–3 mm. apart, directly in photogenic tissue unless otherwise specified. Light emission was detected by a photomultiplier tube modulating one channel of a Tektronix 502 dual beam oscilloscope and photographed together with a second (stimulator) trace. Since all records are of multicellular responses and since no measurements were made of tissue resistance, nominal stimulus voltages have significance only as indications of the relative magnitudes of current flux under different experimental conditions. Further details are given in FF-I.

Action potentials were picked up with electrode pairs of 0.003-inch bare platinum-iridium wire. After amplification by a Grass P-6 amplifier the potentials

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were displayed on the oscilloscope and photographed together with the output signal of the photomultiplier monitoring light emission.

Results

1. *Spontaneous luminescence: multiple flashing*

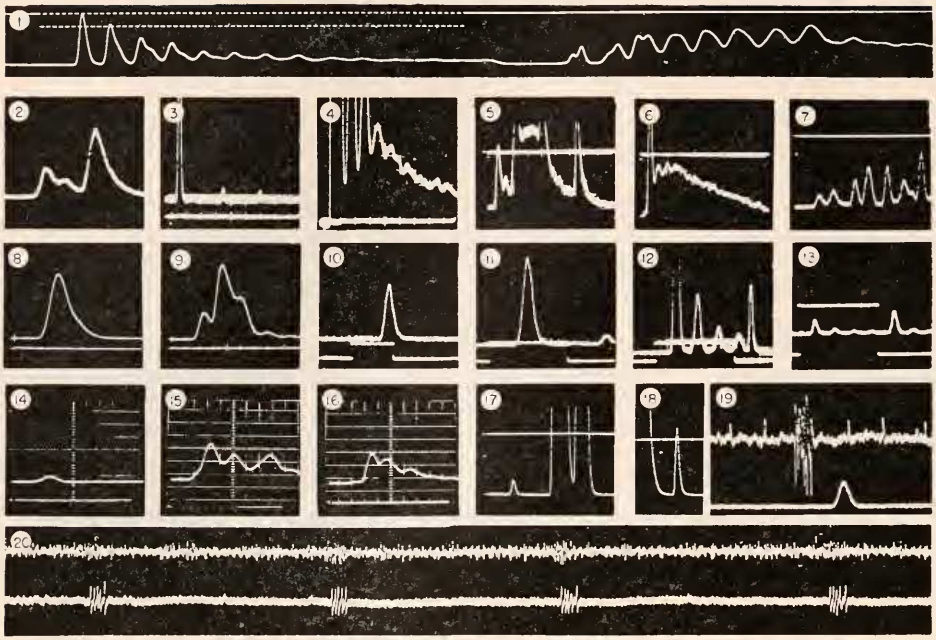
In single-flashing species or individuals successive spontaneous flashes are often very uniform in intensity and frequency, implying a correspondingly regular central nervous signal (*e.g.,* FF-I, Fig. 1). In some species each of the regularly repeated spontaneous flash episodes consists of several partially fused subflashes, implying a more complex excitation. Figure 2 illustrates a triple flash of the male of the Woods Hole *Photuris,* in which the mean inter-peak intervals are roughly 40 and 60 milliseconds (msec.). In the triple flash of the female of the Maryland *Photuris* the inter-peak intervals are both about 85 msec. (Fig. 8 of Hastings and Buck, 1956). Triple flashes with interflash intervals of the order of 60 and 100 msec. were recorded from a small Woods Hole *Photinus* (FF-I, Fig. 7).[2] In most of these instances the intervals between sub-peaks are of the same order as those between successive peaks in the sawtooth luminescence induced by repetitive electrical stimulation at about the limit of 1:1 response (FF-I).

2. *Delayed flashing*

In individuals with central nervous system intact, supernumerary flashing is common after intense stimulation, particularly by trains of such high frequency that the animal has not been able to respond separately to each impulse. For example, in the sequence of *Photuris* responses shown in Figure 1, the major flashes during stimulation at 50 pulses/sec. were at first about 95 msec. apart, or one response to about every fifth stimulus. Some flashes had shoulders indicating a frequency of at least 30/sec., which is considerably above the 20/sec. limit of maintainable 1:1 response to electrical stimulation previously found in this species (FF-I). The flashes soon became less regular and intense and finally merged into a dull glow that died out soon after the stimulus train terminated. Then, more than 300 msec. later, flashing resumed. Like the response during stimulation, this delayed episode showed a major flash frequency of about 10/sec. about four times as high as the maximum rate of spontaneous flashing in intact specimens. Such episodes, each lasting a few hundred milliseconds, may recur at irregular intervals for over five seconds.

The brain is not essential to all post-stimulatory flashing. A decapitated firefly or even an isolated lantern excised with ganglion can often be sent into immediate repetitive activity by a single stimulus after several seconds of rest, and the stimulus need not necessarily be especially intense, particularly if the tissue has been primed by vigorous prior stimulation. For example, in a single segmental organ of *Photuris* that had first been thoroughly aroused by a succession of stimulus trains

[2] Because of a mix-up in the records from two very similar small species of Woods Hole firefly, it is necessary to report that Figure 43, in FF-I, should refer to *Photinus marginellus* and Figure 50 to *Photinus consanguineus,* and that the species identifications for Figures 7 and 49 are uncertain. However, the phenomena illustrated were valid regardless of species responsible.



FIGURES 1–20. (In these oscillographs the time scale is from left to right and is given as S = entire width of picture in milliseconds. Figures identified "As X" refer to the same individual as that of Figure X. Some figures are slightly retouched.) (1) Woods Hole *Photuris,* male, electrodes in head. Responses to train of shocks of 10 m.sec./8 V., 50/sec., with post-stimulatory flashing. S = 2850. (2) Woods Hole *Photuris,* male, intact. Spontaneous flash. S = 270. (3) Iowa *Photuris,* male, isolated lantern. Response to single shock of 1 msec./5 V. S = 1950. (4) As 3, except 1 msec./50 V. Much reduced vertical amplification. S = 1950. (5) *Photinus marginellus,* male, intact. Response to single shock of 5 msec./40 V. S = 5000. (6) As 5, after decapitation. Vertical amplification reduced to 1/5. S = 5000. (7) *Photinus consanguineus,* male, decapitated. Response to single shock of 10 msec./7 V. S = 1000. (8) *Photinus punctulatus,* probably male. Response to single shock of 3 msec./9 V. S = 500. (9) As 8, but 2 msec./40 V. S = 500. (10–12) Woods Hole *Photuris,* male, isolated organ. Responses to single shocks of 100 msec./2.5 V., 200/2.5, and 400/2.5. S = 350, 350, 675. (13) As 10, after further dissection. Response to 400 msec./8 V. S = 675. (14) *Photinus marginellus,* male, decapitated. Response to single shock of 10 msec./15 V. S = 1100. (15) As 14, except 500 msec./30 V. S = 1100. (16) Next response to 15, but to 10 msec./15 V. S = 1100. (17) As 1, but 5 msec./8 V. S = 475. (18) Woods Hole *Photuris,* female, electrodes in head. Response to single shock of 5 msec./5 V. shortly after decay of spontaneous flash. S = 425. (19) Woods Hole *Photuris,* male, intact. Action potential volley from anterior segment of lantern (upper trace) and subsequent spontaneous flash (below). S = 440. (20) Woods Hole *Photuris,* male, intact. Concurrent action potentials from cord between 5th and 6th abdominal ganglia (above) and from surface of 6th segment light organ (below). Four photic volleys included. S = 2150.

close to its limit of 1 : 1 response, a very weak shock sufficed to induce three flashlets after the large primary response (Fig. 3), whereas a stronger one induced a paroxysm of brilliant high-frequency flashing lasting more than 2 seconds (Fig. 4). Figures 5 and 6 illustrate similar irregular luminescence in *P. marginellus* before and after decapitation. Repetitive firing was also induced in *P. consanguineus,* with a major periodicity of about 125 msec. (Fig. 7).

Transition to multiple flashing of a seemingly more regular type than the above can sometimes be induced simply by increasing the strength and/or duration of the stimulus shock. For example, by doubling the voltage the specimen producing the flash shown in Figure 7 was induced to emit a flash like that shown in Figure 9. Similar examples of multipeak flashes in *P. punctulatus* and *P. marginellus* are shown in Figures 12 and 13 of FF-I. Since in such instances there is generally an increase in overall flash intensity, some "new" peaks may represent merely intensification of responses previously too feeble to register. However, Figures 8 and 9, of comparable peak magnitude, show that the additional excitation need not be an amplification artifact. A perhaps analogous instance in the male of *Photuris*, caused by progressively increased stimulus duration, is illustrated in Figures 10, 11, and 12. In Figures 12 and 13 it will be seen that several flashes have intervened, at regular 105-msec. intervals, between the primary responses due to make and break of the current.

Such newly-evoked multipeak responses may maintain their general contour during repeated stimulation so long as stimulus parameters are held constant and stimulus frequency is moderate. It is interesting that the new form may even persist, at least temporarily, after the stimulus is reduced to initial values, indicating lasting facilitation (Figs. 14, 15, 16).

A further suggestion of special cephalic role in stimulation is the fact that voltage, duration or frequency of stimulus, needed to elicit a flash of given magnitude, often increases as the electrode pair is moved progressively forward from abdomen into thorax. When the head is reached, however, parameters may fall to those adequate for stimulating lantern tissue directly.

3. *Central nervous role in response latency*

A. *Cord conduction.* From a large number of measurements on decapitated *Photuris* stimulated at different cord levels, it appears that the typical response delay to anterior thoracic stimulation is in the 110–145-msec. range ("medium latency"). In conjunction with 70–85-msec. latencies to direct lantern stimulation at 22–25° C. (FF-I: Table I and FF-I: Fig. 54), these values suggest cord transit velocities (including junctional delays) of 10–20 cm./sec. for the 7–9-mm. distance. This was confirmed directly in five specimens by making successive anterior thoracic and posterior cord latency measurements on the same individual. There were, however, a few specimens with thoracic latencies of about 90 msec. ("short latency"), indicating cord transit velocities of 40–50 cm./sec., and this also was confirmed by dual-site measurements. Since all determinations were based on numerous measurements and there were apparently no intergrades between the 90 and 110+ msec. latency classes, two excitation modes or pathways are suggested. No bimodal flashes were seen, but since an early flash could blanket a slower response of comparable magnitude (separate flashes are not apparent until paired shocks are 40–50 msec. apart—FF-I) these records do not tell whether short and medium latency responses can occur together.

B. *Brain excitation.* In a search for other indications of dual excitation, intact specimens were stimulated via electrodes in the brain. Some hundreds of such records obtained from five males and three females of *Photuris* seem to fall into two main classes. In one, a single flash of usual intensity occurred with a latency of 120–

150 msec. In the other, the responses involved a very small flash with about a 90-msec. latency and a much brighter and later double flash. These flashes occurred both alone and together in different episodes (Fig. 17). The latency of the large double ("long latency") flash averaged 235 msec. in males and in females seemed to be spread randomly between 500 and 870 msec. after the stimulus (Figs. 21, 22).

The small early element of the response to brain stimulation seems to correspond satisfactorily to the short latency response observed in decapitated photurids, but its characteristic low intensity is unexplained. Occasional atypically large examples can be ascribed to facilitation by a shortly preceding random spontaneous flash (Fig. 18). The 120–150-msec. latency response corresponds to the medium latency response in decapitated specimens. In intact fireflies, therefore, as in decapitated specimens, there appear to be at least two response latencies.

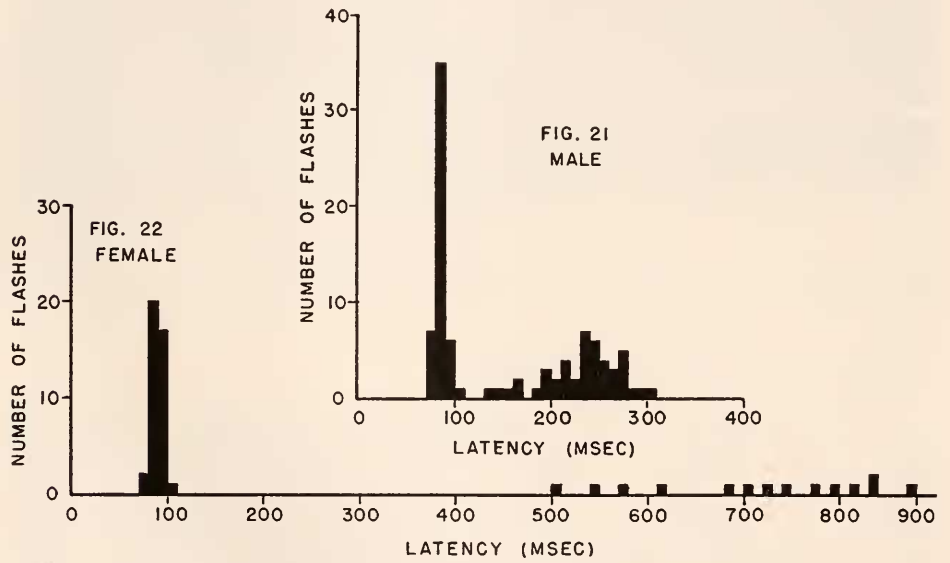


FIGURE 21. Frequency distribution of response latencies to head stimulation in a male of *Photuris*.

FIGURE 22. Frequency distribution of response latencies to head stimulation in a female of *Photuris*.

C. *Central delay*. The long latency response may involve some sort of central delay. This view is favored by the facts that the latency distribution of the late flashes was clearly non-random, at least in the male (Fig. 21). Since the long-latency flashes occurred only in individuals with intact brain-cord connection, they might, alternatively, be ascribed to random endogenous flashing. The relative paucity of flashes in the 0–7- and 100–200-msec. ranges (Fig. 21) could then be due to relative refractoriness of conductor or effector preceding and following the driven flash.

In sum, the roles of brain and cord in excitation seem complex, though some of the response heterogeneity may well reside in lantern tissue. It would clearly be

useful to be able to distinguish spontaneous from driven excitations, if they are different, and to detect directly the excitation signal in lantern or cord. To these ends we attempted to record action potentials associated with luminescence.

4. *Photic action potentials*

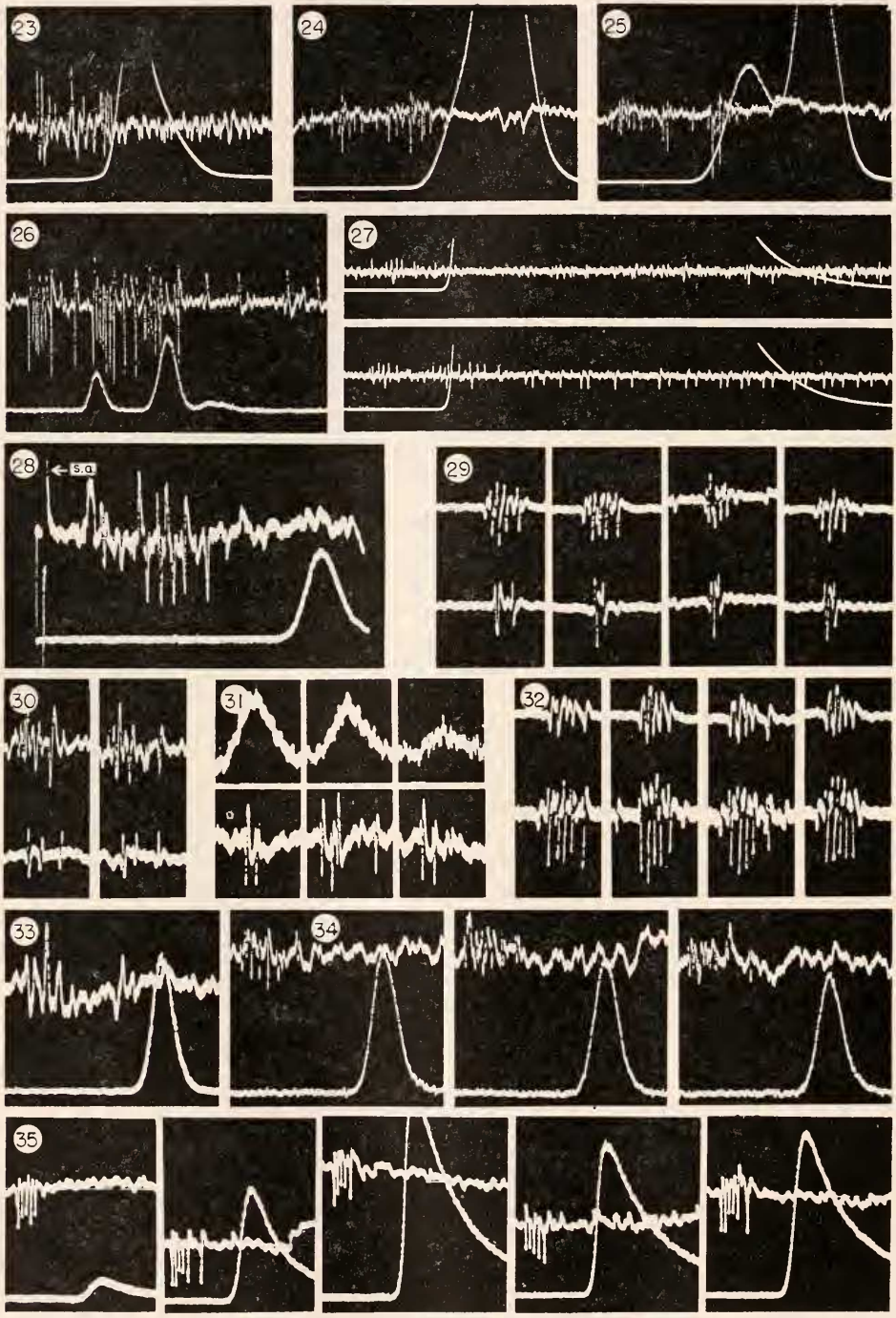
A. *Cord and lantern volleys.* Since *Photuris* is the only firefly among the five species studied that flashes spontaneously with any regularity under laboratory conditions, most of the action potential work was done on this species. Potentials detected in cord lifted free of viscera are much obscured by continuous electrical background which is presumably concerned with musclar activity, but they suffice to demonstrate an unequivocal 1:1 relation between small volleys of nerve spikes and succeeding flashes. Records made directly from photogenic tissue, by laying the electrode pair on regions of the lantern surface that have been stripped of cuticle, exhibit less extraneous electrical activity. The volleys thus detected usually correspond closely in number and spacing to those recorded from cord (Fig. 20) and presumably represent the same excitation signals at a more distal point in their pathway—namely, in peripheral nerve. It is often difficult to say exactly when cord volleys begin, but in most instances they seem to start 5–15 msec. earlier than the corresponding lantern volleys as might be expected from the extra junctional and conduction delays that are presumably incurred by the latter.

It is usually not possible to detect much qualitative agreement in spike patterns between cord volleys and corresponding neutral activity in the lantern, although the volleys appear to be of roughly the same duration. There are occasional suggestions that not every cord volley eventuates in a lantern volley (*e.g.,* between first and second episodes of Figure 20) but this may well be due to the difficulty in distinguishing the photic volley from the non-photic background potentials in the cord.

Usually there is a clear correspondence between gross volley structure and gross flash form (contour). Thus, not only are single volleys typically associated with corresponding single flashes (Figs. 19, 33–35, etc.) but double or bi-partite volleys can apparently slow the accretion phase (Fig. 24) of luminescence and, if sufficiently separate in time, lead to double flashes (Fig. 25). Similarly, triple volleys may induce triple flashes (Fig. 26). In other instances the flash seems to be relatively independent of volley duration *per se,* quite similar flashes often resulting whether the volley ends well before luminescence begins or continues even well past the start of the flash (*e.g.,* Fig. 27).

The interval between the first spike of a spontaneous volley and the rise of the resulting flash was 70–90 msec. in most records from *Photuris* (*e.g.,* Figs. 19, 33–35) and 150–175 msec. in the few records obtained from the Iowa *Photinus pyralis* (Fig. 27), values which are close to the latencies for direct electrical stimulation of the lantern in these species (FF-I: Table I).

After electrical stimulation in the head it was possible to record lantern volleys like those detectable during spontaneous flashing (Fig. 28). The delay between stimulus and first spike was about 60 msec., corresponding to a cord transit velocity of about 13 cm./sec. for an 8 mm. path, and the further delay from first spike to flash was 85 msec., which is within the usual latency range for *Photuris.*



FIGURES 23–35.

Such records thus reinforce the idea that luminescence is excited by neural action potentials.

B. *Generality of Signal.* Simultaneous records from spontaneously flashing specimens were made with two pairs of recording electrodes under two conditions: (a) one pair laterally on the segment 6 organ, the other on the center of the segment 7 organ, (b) one pair on the anteromedial part of 6 and the other on a lateral margin of the same segment of the lantern. In a number of such preparations the volleys from different sites were different in spike number although occurring simultaneously or nearly so (Figs. 29, 30). The number of spikes per volley was in fact somewhat variable even in serial records from the same site and could apparently be as small as two or three (Figs. 29, 30, 31, 35). Furthermore, the signals from a given site could sometimes be altered by slight adjustments in electrode position (Fig. 29 vs. Fig. 32; Fig. 33 vs. Fig. 34a). Thus, until able to record action potentials from a known single peripheral nerve together with only the light from the photocytes controlled by that nerve, we cannot identify the minimal or ultimate light-evoking signal. Yet the fact that one sometimes does get similar volleys from widely separated sites (Fig. 32) suggests that a common excitation signal is widely distributed in the lantern.

C. *Spike pattern in relation to flash intensity and flash contour.* In view of the apparent influence of electrode-tissue relations upon action potential pattern and the fact that all our recordings were made from restrained intact animals capable of at least minor body movements, variation between successive volleys from one site might have little intrinsic significance. But flash intensity is itself known to vary somewhat even during an uninterrupted series of normal spontaneous flashes (FF-1)so it is of interest to see if there is any recognizable relation between spike pattern and the intensity of the associated flash.

Comparisons of volleys preceding flashes of nearly identical intensity given by a single firefly indicated that equal flashes are not always preceded by identical spike patterns (*e.g.*, Fig. 34). Conversely, volleys preceding flashes of differing

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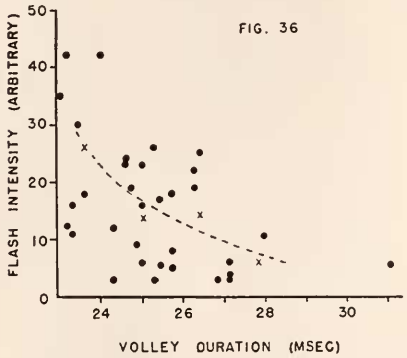
FIGURES 23–35. (23) Iowa *Photuris,* female, intact. Spontaneous flash of both organs and lantern potentials from posterior segment. S = 375. (24) Maryland *Photuris,* sex not recorded, intact. Spontaneous flash and lantern potentials. S = 375. (25) As 24. S = 375. (26) As 19. S = 410. (27) Iowa *Photinus pyralis,* male, intact. Spontaneous flash and associated lantern potentials. Two non-consecutive episodes. S = 1470. (28) Woods Hole *Photuris,* male, intact. Lantern potentials and flash in response to stimulation in head with single shock of 2 msec./6 V. Arrow indicates stimulus artifact (S.A.) S = 240. (29) Woods Hole *Photuris,* male, intact. Action potentials from anterior center of sixth segmental organ (top trace) and lateral edge of same segment (bottom trace) during spontaneous flashing. First, 2nd, 10th and 11th episodes in a series. S = 100 for each episode. (30) Woods Hole *Photuris,* male, intact. Action potentials from 6th segment organ (top trace) and 7th segment organ (bottom trace) of same specimen during spontaneous flashing. Fifth and third episodes in a series. S = 70. (31) As 30, except action potentials (sixth segmental organ) are given with accompanying flashes. To save space, flash records are at high gain and are displaced to left with respect to corresponding volleys. Third, 4th and 5th episodes in a series. S = 70. (32) As 29. Episodes 1, 2, 4, and 7 from one series with electrodes having been slightly readjusted from their positions during recording the series of Figure 29. S = 70. (33) Woods Hole *Photuris,* male, intact. Lantern potentials and spontaneous flash. S = 155. (34) As 33. Seventh, 12th and 13th episodes in a series after slight adjustment of electrode position. S = 155. (35) Woods Hole *Photuris,* female, intact. Lantern potentials and spontaneous flashes. Fifth, 13th, 21st, 22nd and 24th episodes in a series of 33. S = 220, 220, 270, 270, 270.

intensities given by a single individual sometimes look quite similar (*e.g.,* Fig. 35, a, c, and d; b and e). However, each main volley in Figure 35 consists of 5 spikes, and careful measurement of the entire series of 33 volleys indicated that the interval between the first and fifth spikes (duration of main volley) varied inversely with peak flash intensity (Fig. 36). A correlation analysis of these data gave a coefficient of −0.48, showing a highly significant association (< 1% for 32 degrees of freedom). Statistically this means that spike frequency by itself can account for about 25% of the modulation of flash intensity.

It had been found previously that flash intensity in a spontaneous series varies inversely with the interval between flashes (FF-I), which should mean a corresponding inverse relation with the interval between volleys. A plot of flash intensity against the interval between the immediately preceding and second preceding volleys does in fact show such an inverse relation (Fig. 37). The



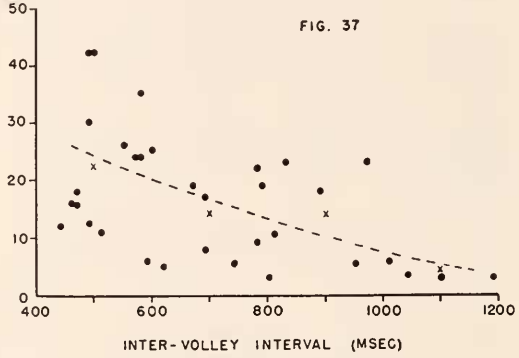


FIGURE 36. Relation between flash intensity and time span of first five spikes in preceding photic volley. Single Woods Hole *Photuris,* female. Crosses indicate means for about 1.4-msec. groupings.

FIGURE 37. Relation between flash intensity and interval between immediately preceding and second preceding photic volleys. Same Woods Hole *Photuris,* female, as in Figure 36. Crosses indicate means for 200-msec. groupings.

correlation coefficient is −0.50 which is significant at the 1% level. Thus, volley frequency by itself also can account for about 25% of the intensity modulation.

A plot of volley duration for a given episode against the intervolley interval preceding the respective flash indicates association of the two neural variables. The correlation coefficient is 0.53, again highly significant. Spike frequency thus tends to rise as volley frequency rises. The multiple correlation coefficient for flash intensity as influenced by both volley duration and volley frequency is −0.56. This means that because of the high correlation of spike frequency with volley frequency, the combined and approximately equal effects due to these two sources of stimulatory facilitation account for only 31% of the overall influences on flash intensity. Presumably much of the remaining variation is due to non-correspondence of the conductor and effector populations recorded, but there is no reason to exclude the possibility also of modulation at the level of photocyte or neuroeffector junction.

The obvious association of gross volley structure and gross flash form (Figs. 25, 26) suggests that detailed spike distribution might be related to more subtle differences in flash form, such as between symmetrical (Figs. 19, 28, 33, 34, 38, 39) and asymmetrical (Figs. 23, 35) flashes, but in fact even the volleys produced by single individuals of *Photuris* emitting symmetrical flashes show much variation (Figs. 33, 34).

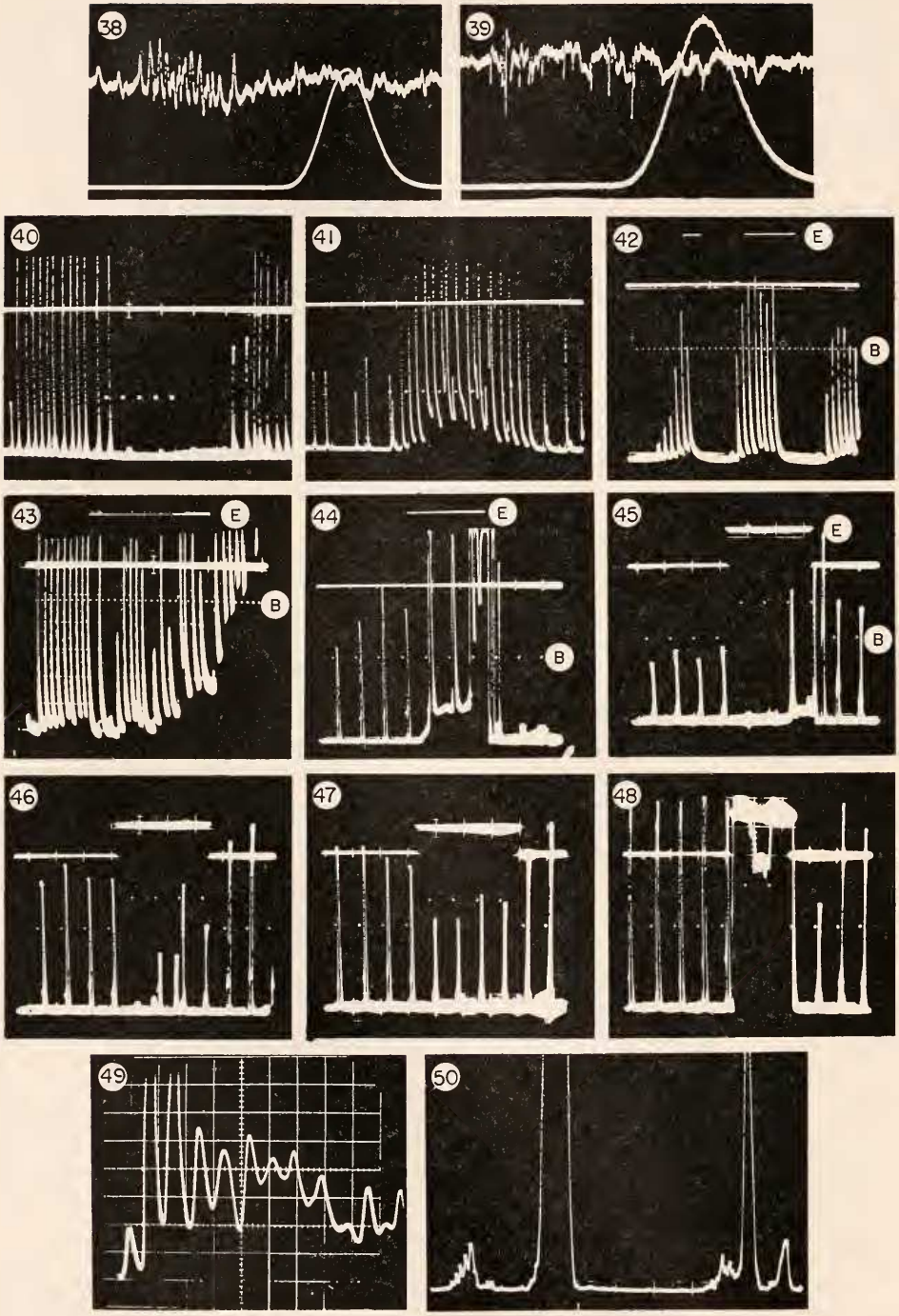
D. *Inter-volley electrical activity.* Isolated single spikes commonly occur throughout the flashing cycle, usually irregularly but sometimes quite regularly (Figs. 19, 27). In our preliminary accounts we suggested that these inter-volley spikes might have a trophic function in sustaining lantern excitability between volleys. Long serial records now have shown, however, that these potentials normally tend to occur in groups lasting 1–1.5 seconds and recurring every 2 to 3.5 seconds, quite independently of the photic volleys. Furthermore, the isolated spikes have a consistently different wave form from spikes in photic volleys. The supposition, therefore, is that they have to do with some other cyclic function, for example, spiracular control. Dr. Albert Carlson (personal communication) has found that the amount of intervolley noise is considerably reduced without affecting the photic volleys if the surface of the lantern is allowed to dry slightly.

5. *Experimental inhibition of luminescence*

The ease with which flashing can be induced when electrodes are inserted in the brain, and the possible express conduction of cephalic stimulation, raised the question of whether specific centers for the excitation of luminescence are present. As a control we therefore tried stimulating an intact animal via electrodes in the eye, which was presumed to be an indifferent cephalic site. To our surprise such stimulation, far from inducing luminescence, sometimes actually suppressed spontaneous flashing. Figure 40 shows an example in which five stimuli one second apart produced an immediate inhibition which lasted for four seconds after eye stimulation ceased. Stimulation by electrodes in the brain of the same animal, on the contrary, enhanced flashing (Fig. 41). Further, stimulation in the eye could also suppress, sometimes after a delay, response of the lantern to stimulation via a second pair of electrodes in the brain (Fig. 42). In such experiments the site of electrode placement in the eye and the relative stimulation frequencies and voltages to eye and brain are apparently quite critical, since partial or complete failure of inhibition by eye stimulation (Figs. 43, 45) or even enhancement of flash intensity (Fig. 44) were observed under some circumstances. Spontaneous flashing, likewise, can either (a) be inhibited essentially completely (Fig. 40), (b) escape from the inhibition after a time even though eye stimulation continues, or (c) not be markedly affected, depending on voltage. An analogous effect, seen with direct current applied to the eye during serial stimulation of the brain, seems to indicate that the response can be quantitatively modified, depending rather critically on relative voltages at the two pairs of electrodes (Figs. 45–48). Details of this inhibition will be considered in another communication.

6. *Effect of eserine*

A number of agents (spider venom, hypoxia, cyanide, ether, etc.) are known to upset effector co-ordination and bring about asynchronous lighting of minute



FIGURES 38–50.

areas of the lantern ("scintillation"). We will consider the morphological nature of the small luminous units elsewhere: for present purposes it suffices to say that they comprise both single photocytes and small aggregations. We found that 10^{-3} and 10^{-4} *M* eserine perfused through the abdomen also induces scintillation. The frequency of firing (13–17/sec.—Fig. 49) was close to the limit of 1:1 response to train stimulation (FF-I). In some preparations the eserine effect was particularly dramatic in that the bouts of sparkling alternated regularly with intervals of near darkness (Fig. 50). Since scintillation is a nonspecific response there is no compelling reason to homologize the action of eserine in fireflies with that in better known systems but the relatively sudden onset of the paroxysms of luminescence and their equally sudden quenching bear a remarkable resemblance, for example, to the activity-block cycles of post-synaptic elements of eserinized cockroach cercal ganglia (Roeder *et al.*, 1947).

The drug was ineffective in inducing scintillation in a deganglionated light organ, but since the photogenic tissue is well insulated from the hemocoel by the "reflector" layer of the lantern it is not certain that peripheral junctions were actually exposed.

Intensive efforts to record potentials from lanterns of eserinized fireflies both intact and decapitated did not yield any information about unit activity.

7. *Excitatory state*

Specimens often show very low threshold for electrically-induced flashing when first mounted with electrodes, the threshold then rapidly rising to a higher level which remains stable for an hour or more. Whether the initial condition is one of hyperexcitability induced by handling or the later state one of adaptation is unknown but for reproducible records we customarily waited about 15 minutes after first mounting the specimen.

Fireflies are occasionally refractory. This refractoriness is manifested by inordinately high electrical thresholds, dim induced luminescence, lack of spontaneous flashing and lethargic behavior. It seems not clearly correlated with time of day, but this is not necessarily conclusive because of the irregular illumination regimen of most specimens over their usual several days of laboratory life. Full responsiveness can usually be restored by handling or other irritation. Scratching the head with a needle, particularly if the cuticle is broken, is usually effective, as is

FIGURES 38–50. (38) Maryland *Photuris,* probably male, intact. Lantern potentials and spontaneous flash. S = 300. (39) Maryland *Photuris,* probably male, lantern potentials and spontaneous flash. S = 300. (40) Woods Hole *Photuris,* male, intact. Spontaneous flashing. The five dots denote stimuli to eye, 15 msec./10 V., 1/sec. S = 18 sec. (41) As 40. Stimulator trace: train of six shocks to brain of 15 msec./10 V., 1/sec. S = 18 sec. (42) Woods Hole *Photuris,* male, intact. Top stimulator trace (E), two trains of stimuli to eye, 4 msec./10 V., 30/sec. Lower stimulator trace (B), stimuli to brain of 2 msec./12 V., 2/sec. S = 21 sec. (43) As 42. Top trace (E): train of stimuli to eye of 4 msec./10 V., 10/sec. Lower trace (B): brain stimulation at 4 msec./7 V., 2/sec. S = 21 sec. (44) Woods Hole *Photuris,* female, intact. Top trace (E): stimuli to eye of 5 msec./15 V., 40/sec. Lower trace (B): brain stimuli of 3 msec./14 V., 1/2 sec. S = 21 sec. (45–48) As 44 except eye stimuli are, respectively, 7 V. DC, 6 V. DC, 5 V. DC and 8 V. DC. S = 21 sec. (49) Iowa *Photuris,* sex not recorded; 10^{-3} *M* eserine in hemocoel. Flash frequency about 13/sec. S = 1150. (50) Iowa *Photinus pyralis,* male, luminescence of isolated organ after hemocoel injection of 10^{-4} *M* eserine. S = 5 sec.

chasing the animal around on the table top for a minute or so before it is fastened down for experimentation. It is significant that any arousing has to be done before decapitation or cord section, as if the state of the central nervous system determines the responsiveness of the whole excitation pathway.

In addition to involving a transition from a motionless individual, standing with bowed head, to an actively walking firefly with antennae waving, the "arousal syndrome" has interesting luminescent manifestations. As the animal becomes disturbed the originally dark lantern begins to show dim irregular local flecks or blotches of light which grow progressively. Eventually the whole lantern may blush dimly and then finally emit a bright flash followed by total extinction. After this, normal spontaneous flashing can occur.

Discussion

Both voluntary and electrically-induced flashes are invariably preceded by characteristic volleys of action potentials which can be detected in the cord and in peripheral nerve within lantern tissue. This evidence, along with Hanson's (1962) experiments on the gross conduction pathways of the lantern, demonstrates directly for the first time that the control of bioluminescence can be along conventional neuroeffector lines.[3] We have also observed a variety of spontaneous and induced luminescent phenomena which, in spite of the limitations of our extracellular recordings, the present unavailability of unit conductor-effector preparations, and our ignorance of modulating potentialities of the effector tissue itself, suggest the following further details of central nervous involvement in flash control.

1. *The excitation signal*

The rhythmicity of spontaneous flashing, its cessation upon decapitation, and the low threshold to cephalic stimulation point to the brain as the normal trigger for flashing and the site of a pacemaker of remarkable regularity. Volleys clean enough for detailed analysis could not be recorded even from the posterior part of the cord but it is a reasonable postulate that both spontaneous and electrically induced flashing depend on central generation and propagation of volleys similar to those recorded from peripheral nerve. This signal is specific in its close sequential association with flashing and with no other visible activity. Its gross similarity in cord and lantern and its nearly simultaneous arrival in different parts of the luminous tissue point to a general excitation of the lantern.

The statistically demonstrable modulation of flash intensity by the photic volley

[3] In some Japanese and Korean fireflies light is not emitted as brief flashes separated by complete darkness, but as a long-lasting glow fluctuating slowly between bright and dim. Hasama, in a series of studies on such species (*e.g.*, Hasama 1939, 1942), figures monophasic "action potentials" detected with paired non-polarizable wick electrodes, one resting on the external cuticle of a non-luminous segment, the other on a luminous segment (said to be more negative). These string galvanometer records, which consist of rhythmic sawtooths with a period of 5–6 seconds, were said to correspond "fast ganz" to the frequency of light emission. The latter was not recorded but was said to be 15–20/sec. The potentials were reported not to correspond to the ventilatory rhythm but were found to be temporarily enhanced in magnitude, and slightly in frequency, by oxygen. The relation between Hasama's potentials and ours is uncertain, but it seems clear that the Japanese worker cannot have been dealing with conventional nervous action potentials.

shows effector facilitation by the action potential spikes, and also by volley frequency. There is often a rough correspondence between spike groupings in multiple volleys and the general form of the corresponding flash but the finer details of single flash contour are apparently not associated directly with specific spike patterns within the volley. Rather it seems likely that the time course of luminescence during spontaneous flashes—for example whether the contour is symmetrical or the rise more rapid than decay—depends primarily on the response characteristics of the effector cells or upon the topographic distribution of excitation pathways (Buck, 1955).

2. *Latency, central delay and conduction velocity*

Measurements on intact and decapitated specimens of *Photuris* stimulated at the anterior end of the cord indicate three head-to-lantern latency classes. The head-dependent, long-delayed (200+ msec.) flash is ascribed tentatively to central after-discharge. The short (ca. 90 msec.) and medium (ca. 120 msec.) latencies might reflect excitation via one or the other of two pathways conducting at different velocities, the variability in excitation being perhaps related to the crudeness of the stimulating electrodes and their variable placement. Alternatively, the longer latency might be due to delay in brain or cord. Unfortunately our action potential records are of little help in choosing between dual pathways and central delay, because only one stimulus-to-volley latency class, corresponding to the slower conduction velocity, is present in the few records that show artifact, volley and flash together.

Behavioral data from *Photinus pyralis* demonstrate another ambiguity of central nervous latency. In the nuptial signaling of this species the male cruises about, flashing every 5.8 seconds (at 25°). The perched female does not flash except in response to a male that flies within 20 feet or so of her, in which case she replies to his flash after an interval of about 2 seconds. Now, the reaction time of the female is fairly precise—in fact this is the crucial cue that enables the male to distinguish her flash from those of other males (Buck, 1937b). Hence the 2000 msec. could very properly be called the latency of her presumably reflex response to ocular stimulation, even though it is obviously quite a different kind of latency from the 200–300 msec. that would presumably be measured for flashing in response to strong electrical stimulation of the eye in this species.[4] (It is interesting, incidentally, that the female's flash is triggered by a stimulus (light) that would be strongly inhibitory were it somewhat more intense.)

3. *Types of endogenous rhythm*

Fireflies exhibit several types of rhythmic or repetitively patterned luminescence. Insofar as the potentialities of the central nervous system in exciting such flashing are concerned there is probably no intrinsic reason for excluding the high frequency flashing that occurs only during or shortly after vigorous stimulation (Fig. 1, 4–7) or the apparently ganglion-dependent pulsing luminescence of eserinized fireflies (Figs. 49, 50). Similarly it seems very likely that the regular

[4] Estimated on basis of the 150-msec. direct lantern latency (FF-I, Table I) plus 100 msec. for cord transit.

multipeak flashes induced electrically (Figs. 9, 15) involve much the same excitation process and sequence as compound flashes that occur without any obvious external stimulation (Fig. 2). There is, however, some conceptual simplification in restricting the consideration of rhythm to "normal" or "spontaneous" manifestations. These include the repetitive flashing during flight, the production of multipeak flashes and the inherent diurnal activity rhythm.

The head-dependent, highly species-characteristic and often intricate flashing patterns of flying males of many New World lampyrid fireflies illustrate the precision of programming attained by the central nervous system. These signals are not only regularly repeated usually at intervals of several seconds, but may display a remarkable constancy of timing and relative intensities of sub-flashes or peaks within each flashing episode (McDermott, 1914; McDermott and Buck, 1959). Previous studies have also shown that this spontaneous rhythm is temperature-dependent to a degree similar to those of insect neuromuscular activities such as ventilation (Snyder and Snyder, 1920; Buck, 1937b).

Compound flashes themselves illustrate another type of repetitive luminescence that is possibly dependent on the central nervous system. Three mechanisms seem possible: (1) sub-peaks could be due to synchronous repetitive firing of photocytes in response to repetitive volleys from the central nervous system (*e.g.*, Fig. 26). The fact that similar flashes can be elicited from isolated lanterns indicates that excitation need not involve more than one or two ganglia of the cord. (2) The sub-peaks could represent responses of photocyte populations differing in latency. The oscilloscope record would look the same whether these populations were spatially separate or intermingled, and because of the high frequency of the repetitive flashing in each episode it would usually not be possible to distinguish visually between these alternatives. However, on rare occasions it appears that the two segmental organs or separate areas of one segment may flash slightly out of phase with each other. (3) The sub-flashes could reflect a conductional pattern leading to asynchronous excitation of different photocyte populations (Buck, 1955).

The 24-hour cycle of flashing activity is little known but is presumably controlled by a still different pacemaker mechanism from the above. This, in contrast to numerous other biological clocks, seems to vary with mid-range temperature (Mather, 1947). Curiously, also, this long-period cycling is inhibited by light intensities both above and below a relatively narrow range of ambient values (Buck, 1937a). Hence, like the modifications in the species flash pattern that occur during the mating signals, the pacemaker is responsive to sensory input.

4. *Excitatory state*

Many qualitative and quantitative characteristics of flashing are reasonably stable over at least limited periods and vary predictably and directly with stimulus parameters (FF-I). There is also much evidence that the whole level of excitability may become high, as in the initial low threshold period and in post-stimulatory luminescence, or low, as in the examples of refractoriness described. The involvement of the central nervous system in some of these phenomena is indicated by the necessity for an intact head-cord connection during arousal and in the restriction of delayed post-stimulatory flashing to intact specimens. Further, Carlson (1961) found that even such an ostensibly involuntary or automatic re-

sponse as the "pseudoflash," evoked in hypoxic fireflies by sudden readmission of oxygen, is reduced during refractory periods of the diurnal cycle unless the animal is aroused before subjection to hypoxia. It is interesting, in this connection, that the pseudoflash can only be induced at a stage of hypoxia so severe that the peripheral nerves are electrically silent. Hence it appears that the necessary degree of peripheral facilitation depends on prior central activity. In regard to inhibition, also, our experiments with electrical stimulation via the eye at least suggest the possibility of an interplay between stimulatory and inhibitory centers in the brain.

The question of excitation in the post-ganglionic portion of the excitation pathway will be considered in the third paper of this series.

Summary

1. The central nervous system is shown to be involved in (a) normal spontaneous flashing, both single and multiple, (b) some types of post-stimulatory flashing and scintillation, (c) comatose behavior and refractoriness to stimulation.

2. Two and probably three latencies in response to head and anterior cord stimulation exist. At present it is not possible to distinguish between cord pathways (conducting at ca. 15 and 50 cm./sec.) and central delay as possible causes of these latency differences.

3. From posterior cord and from lantern surface it is possible to record small and characteristic volleys of action potentials associated 1 : 1 with spontaneous flashing and involving latencies comparable with those previously found for electrical stimulation. Multiple volleys may invoke multiple flashes. Flash intensity increases with both volley frequency and spike frequency but there is apparently not a close relation between volley structure and flash contour.

4. Electrical stimulation in the eye can either inhibit or enhance flashing, depending on relative intensities of brain and eye stimulation.

5. In preparations including ganglia the anti-cholinesterase eserine can induce both asynchronous activation of small units and a recurrent alternating large scale activation and block of luminescence.

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